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Effects of genotype and salting time on chemical, physical and sensorial traits of a new pig seasoned meat product 'Cuore Di Spalla'

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ABSTRACT

The aim of this work was to determine the chemical, physical and sensorial characteristics of a cured product called 'Cuore di Spalla', resulting from two different pig genotypes (G) and two salting times (S). Fourteen Duroc × Large White (D × LW) and 14 Cinta Senese × Large White (CS × LW), reared in the same condition in outdoor and fed commercial mixture. The animals were slaughtered at 10 months of age which corresponds to an average live weight of 143 ± 15 kg for D × LW and 122 ± 15 kg for CS × LW. The right shoulders were salted for 3 days (L) and the left ones for 5 days (H). Physical (colour, texture profile analysis), chemical (moisture; crude protein; IMF; ash; salt; total lipids; fatty acids) and sensorial analyses (by panellists' group) as well as volatile compounds profile (by SPME-GC-MS technique) were determined at the end of seasoning period. The genotype affected product's size (1484.3 vs 1857.1 g of final weight for CS × LW and D × LW respectively), chemical characteristics and texture (cohesiveness 0.50 vs 0.53 and springiness 6.73 vs 7.12 for CS × LW and D × LW respectively). Aromatic profile was less affected by the factors considered or at least the panellists were not able to discriminate the effect. The main family of volatile compounds affected by genotype was alcohols. Salting time seemed to affect only the parameters closely related to salt (16.52 vs 15.33% of salt and 5.15 vs 4.35 for saltiness in H vs L respectively).

HIGHLIGHTS

- The genotypes determined differences in size, fat quantity and physical-chemical parameters of the 'Cuore di Spalla' product.
- Very limited differences in the aromatic profile were found.
- Both salting times led to high salt content in the product.
- It seems feasible to produce the 'Cuore di Spalla' with further reduction of salting times.

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Genotype; salt content; pig; meat quality; flavour

Introduction

Local pigs are generally linked to products characterised by high specificity. Various researches pinpointed the differences between products coming from autochthonous pigs compared to improved ones (Franci et al. 2007; Sirtori et al. 2011; Pugliese and Sirtori 2012) and the genetic effect on meat quality is recognised. Mediterranean local pigs are often crossed with selected breeds, as in the production of Duroc × Iberian (Fuentes et al. 2014) and Large White × Corsican pigs (Coutron-Gambotti et al. 1998). In Italy, historically, crosses between Cinta Senese sows and Large White boars have been produced by Cinta Senese farmers (Pugliese and Sirtori 2012).

The crossbreeding counterbalance some limits of autochthonous pigs both on *in vita* performances and on carcase and meat traits (Sirtori et al. 2011).

The use of local breeds to produce dry-cured meats must take into account the qualitative characteristics of the raw material. The greater adipogenicity of the local breeds influences the final characteristics of the products (Sirtori et al. 2011) as well as the production process, including the diffusion of salt.

The dry-cured products produced with the autochthonous breeds are often characterised by high salt content (Hersleth et al. 2011); the highest moisture and the lowest size characterising the products of these breeds favour the penetration of salt. In

addition, especially in southern Europe, the traditional seasoning methods are characterised by excessive use of salt (Pugliese and Sirtori 2012), generating urgent worries for human health. Recently, European Union (EU) was forced to implement salt reduction initiatives because of its negative effect on hypertension (Corral et al. 2013). However, salt cannot be reduced without considering its influence on the sensorial and technologic characteristics (Corral et al. 2014); like the development of an optimal texture through proteolysis (Purriños et al. 2012; Harkouss et al. 2015) and the formation of flavours and aromas due to its lipids pro-oxidant role during curing (Andrés et al. 2002; Purriños et al. 2012). In dry-cured products the penetration of salt is pivotal to ensure product's safety and stability, as well as to develop characteristic sensory attributes (Martuscelli et al. 2017). The NaCl contributes to control the endogenous enzymatic activity and is responsible for the salty taste and typical texture of dry-cured ham (Flores et al. 2012). The curing process consists of a stabilisation phase at low temperature, that includes the curing and resting steps and a further phase of drying-ageing at increasing temperatures. The main goal of the stabilisation phase is to reduce water activity (a_w), thus increasing salt and decreasing water contents (Garcia-Gil et al. 2012). The amount of salt used can be undetermined or exact. The first procedure is mainly used in Spain and part of France and Italy and the product is completely covered by salt for several days. In the second way the exact amount of salt per kg of fresh ham is added on the lean surface and hand-rubbed (Toldrá 2002). Other factors can affect the penetration of the salt in meat; primarily the characteristics of the raw product such as size, moisture, fat, as well as if the product is whole or partitioned (Sánchez-Molinero and Arnau 2008; Gou et al. 2008). These factors affect the diffusion process, where the salt absorption is parallel to a water loss caused by differences in concentration and osmotic pressures (Raoult-Wack 1994).

The objective of this study was to evaluate the influence of two genotypes and two different salting times on technological, physical and chemical traits as well as on the volatile compounds (VOCs) profile of a dry-cured pig product.

Materials and methods

Samples manufacturing

Twenty-eight castrated male pigs, 14 belonging to Duroc × Large White ($D \times LW$) and 14 to Cinta Senese × Large White ($CS \times LW$) genotypes, were

reared in the same conditions. The animals were slaughtered at the same age to an average live weight of 143 ± 15 kg for $D \times LW$ and 122 ± 15 kg for $CS \times LW$. From the boneless shoulder, including all muscles and removing subcutaneous fat, the product called 'Cuore di Spalla' was created. The right shoulder of each pig was salted for 3 days (Low salt: L) while the left shoulder for 5 days (High salt: H). The dry-cured period was carried out in a controlled cell for humidity and temperature. After salting, the shoulders were washed, stuffed inside a synthetic casing and rested in a chamber under controlled atmosphere (at 4–6 °C and 70–85% relative humidity) for 4 weeks. Subsequently, the shoulders were dried at 14–20 °C and 60–80% RH for 5 months, approximately. The authors declare that the animals were bred in 'Le Selve di Vallolmo' farm, following the principles of the declaration of Helsinki and the experiments complied laboratory health and safety procedures.

Colour and chemical analysis

At the end of seasoning time, colour parameters CIE L^* (lightness), a (redness) and b (yellowness) were measured on a slice of each product, repeated four times, using a Minolta colourimeter CR-200 (Minolta Camera Co., Ltd, Osaka, Japan). Recalibration on white and red plates was performed at the start of each measuring session.

Chemical analyses were performed according to AOAC methods (AOAC 2000) on samples deriving from the whole ground product: (i) moisture by lyophilising to a constant weight; (ii) intramuscular fat (IMF) as ether extract; and (iii) protein using the Kjeldahl method.

Texture profile analysis (TPA)

Texture Profile Analysis (TPA) were carried out on 20-mm-thick lean slices cut and accurately carved with a scalpel into 3 cubes of 10 mm per side (dimensions of 10 mm × 10 mm × 10 mm for length, width and height, respectively) for each sample. TPA was performed using a texture analyser (Zwick GmbH & Co. KG, Ulm, Germany) with a 1 kN-load cell and a 100-mm-diameter compression plate (modification of the method described by Morales et al. 2007). The samples were compressed twice to 50% of their original height (time = 0 s between the two compression cycles), at a crosshead speed of 1 mm/s and perpendicularly to the fibre-bundle direction. Force–time curves were recorded, and the following parameters were

calculated, according to Ruiz-Ramirez et al. (2005): hardness (N), adhesiveness (J), cohesiveness (dimensionless), springiness (mm) and chewiness (J).

Fatty acid composition

Total lipids content was determined using the method of Folch et al. (1957); fatty acid profile of total lipids, using the modified technique of Morrison and Smith (1964). Fatty acids (FAs) methyl esters were analysed by gas chromatography using a Varian 430 apparatus (Varian Inc., Palo Alto, CA, USA) equipped with a flame ionisation detector. FAs separation occurred in a Supelco Omegawax TM 320 capillary column (30-m length; 0.32 mm internal diameter; 0.25 µm film thickness; Supelco, Bellefonte, PA, USA). The chromatographic conditions were an initial temperature of 160 °C, which was then increased by 2 °C/min until the temperature reached 220 °C. One microliter of sample in hexane was injected with the carrier gas (helium) at a constant flow of 1.5 mL min⁻¹ and at a split ratio of 1:20. The detector temperature was set at 260 °C. The chromatograms were recorded using computing integrator software (Galaxie Chromatography Data System 1.9.302.952; Varian Inc.). The FAs were identified by comparing the retention time of the FAME with the standard Supelco 37-component FAME mix (Supelco) and were quantified through calibration curves using nonadecanoic acid (C19:0) (Supelco) as an internal standard. Results were expressed as grams of FAs per 100 g of dry product.

Analysis of volatile compounds

The VOCs profile was obtained by SPME-GC-MS technique. An Agilent 7890 GC-chromatograph equipped with a 5975A MSD with EI ionisation was used for analysis. A three-phase DVB/Carboxen/PDMS 75-µm SPME fibre, (Supelco, Bellefonte, PA, USA) was exposed in the head space of the vials at 60 °C for 30 min for volatile compound sampling after 5-min equilibration time. A Gerstel MPS2 XL autosampler equipped with magnetic transportation adapter and a temperature controlled agitator (250 rpm with on/cycles of 10 s) was used for ensuring consistent SPME extraction conditions. Chromatographic conditions were column J&W Innovax 30 m, 0.25 mm, ID 0.5 µm DF; injection temperature 250 °C, splitless mode, oven programme 40 °C for 1 min then 2 °C/min to 60 °C, then 3 °C/min to 150 °C, then 10 °C/min to 200 °C, then 25 °C/min to 260 °C for 6.6 min. Mass spectra were acquired within the M/Z interval 29–350 with an Agilent 5975C MSD

spectrometer at a scan speed such to obtain three scans/s. After acquisition, the GC-MS chromatogram was processed by a CLEAR VIEW™ (Markes, UK) algorithm to dynamically subtract the background noise contribution along the chromatograms, defining an average chromatographic peak width of 4 s. VOCs were identified by matching EI mass spectra against NIST 05 or Wiley 07 spectral library and Kovats indices. The peak area of each compound was determined on a specific target ion and confirmed by the matching of two qualifier ions using a maximum threshold ratio of 20%. The peak areas for each analyte were normalised for the appropriate internal standard. A detailed description of the method can be found in Pugliese et al. (2010).

Sensory analysis

Two slices, 2 mm thick, for each cured shoulder were evaluated by a trained panel of 10 members using a descriptive analysis method: 12 traits regarding sensory characteristics, grouped in appearance (visual intramuscular fat, colour intensity and oiliness), texture (hardness), aroma (aroma and after-flavour) and taste (saltiness, after-taste, rancid, chewiness, juiciness and persistence) were evaluated. A 10-cm unstructured scale was used, whose extremes were 'very low' and 'very high'. All sessions were done at 20–24 °C in a panel room equipped with fluorescent lighting. Four products were evaluated simultaneously assessing the four treatment combinations (H-CS × LW; L-CS × LW; H-D × LW; L-D × LW) per session with a total of 14 sessions. Samples order was randomised.

Statistical analysis

Data were analysed using the GLM procedure (SAS 2012) using genotype, salting time and subject as discrete effects:

$$Y_{ijkl} = \mu + G_i + S_j + N_k(G_i) + (G \times S)_{ij} + E_{ijkl}$$

For sensorial data the following model was used:

$$Y_{ijklm} = \mu + G_i + S_j + N_k(G_i) + (G \times S)_{ij} + T_l + E_{ijklm}$$

Where G = genotype effect (i = 1,2); S = salting time effect (j = 1,2); N = subject effect; T = evaluator; E = error effect.

The genotype effect was tested against subject within genotype in both statistical analyses. The residual mean square was used as the error term for the effect of salting time.

Results

Size, colour, chemical traits and texture profile analysis (TPA)

Genotype strongly influenced almost all parameters (Table 1). D × LW products were heavier both at the beginning and at the end of seasoning ($p < .01$), while seasoning loss ($p > .05$) was not affected. As regards chemical traits, CS × LW showed the highest moisture ($p < .05$) while no difference was recorded in crude protein percentage. D × LW pigs recorded higher percentage of IMF ($p < .01$) and lower of Ash and Salt ($p < .01$) than CS × LW. Colour parameters were higher in D × LW for all the three coordinates.

The salting time seems to have affected only salt content, being higher in the H group, and consequently also ash ($p < .05$). This result mainly concerns the D × LW genotype within which the difference between H and L was statistically significant (14.8 vs 11.9%).

TPA analysis (Table 2) showed higher values of Cohesiveness ($p < .05$) and Springiness ($p < .01$) in D × LW than CS × LW. Salting Time did not affect any parameters.

Fatty acid composition

The results of FAs profile are shown in Table 3. D × LW product showed the highest quantity of total

Table 1. Technological and chemical traits and colour parameters of 'Cuore di spalla' as influenced by genotype and salting time.

	Genotype		<i>p</i> -value	Salting time		<i>p</i> -value	G × S <i>p</i> -value	RSME
	CS × LW	D × LW		H	L			
Initial weight, g	2500.00	3098.10	**	2833.00	2766.20	ns	ns	161.00
Final weight, g	1484.30	1857.10	**	1688.60	1652.80	ns	ns	156.20
Seasoning loss, %	40.85	40.23	ns	40.66	40.42	ns	ns	2.69
Moisture, %	47.12	45.25	*	45.80	46.57	ns	ns	2.97
Crude protein, %	59.92	58.24	ns	58.72	59.44	ns	ns	2.27
Ether extract, %	17.92	24.49	**	20.93	21.48	ns	ns	1.69
Ash, %	21.10	16.77	**	19.58	18.29	*	ns	2.51
Salt, %	18.43	13.43	**	16.52	15.33	*	*	2.65
L*	36.23	40.02	*	37.99	38.45	ns	ns	1.92
a	16.10	17.81	*	16.51	17.40	ns	ns	1.73
b	4.90	6.67	*	5.84	5.73	ns	ns	2.27

CS × LW: Cinta Senese × Large White; D × LW: Duroc × Large White; H: 5 days of salting time; L: 3 days of salting time.

Chemical analyses are reported on dry matter.

* $p < .05$; ** $p < .01$; ns: not significant.

Table 2. Texture profile of lean of 'Cuore di spalla' as influenced by genotype and salting time.

	Genotype		<i>p</i> -value	Salting time		<i>p</i> -value	G × S <i>p</i> -value	RMSE
	CS × LW	D × LW		H	L			
Hardness, N	9.53	11.02	ns	11.37	9.17	ns	ns	5.32
Cohesiveness	0.50	0.53	*	0.51	0.52	ns	ns	0.04
Springiness, mm	6.73	7.12	*	6.96	6.89	ns	ns	0.23
Chewiness, N*mm	30.02	22.52	ns	28.35	24.20	ns	ns	11.41

CS × LW: Cinta Senese × Large White; D × LW: Duroc × Large White; H: 5 days of salting time; L: 3 days of salting time.

* $p < .05$; ** $p < .01$; ns: not significant.

Table 3. Lipid content and fatty acid composition of 'Cuore di spalla' as influenced by genotype and salting time (g/100 g of dry matter).

	Genotype		<i>p</i> -value	Salting time		<i>p</i> -value	G × S <i>p</i> -value	RMSE
	CS × LW	D × LW		H	L			
Total lipid	18.32	23.49	**	21.22	20.59	ns	ns	3.21
C16:0	4.40	5.69	**	5.16	4.94	ns	ns	0.79
C16:1	0.60	0.77	**	0.69	0.68	ns	ns	0.11
C18:0	1.78	2.47	**	2.16	2.09	ns	ns	0.32
C18:1	6.43	8.64	**	7.63	7.45	ns	ns	1.14
C18:2	1.03	1.46	**	1.24	1.26	ns	ns	0.24
C18:3	0.04	0.07	**	0.05	0.06	ns	ns	0.01
SFA	6.55	8.64	**	7.75	7.44	ns	ns	1.17
MUFA	7.20	9.59	**	8.50	8.30	ns	ns	1.26
PUFA	1.16	1.63	**	1.38	1.41	ns	ns	0.27

CS × LW: Cinta Senese × Large White; D × LW: Duroc × Large White; H: 5 days of salting time; L: 3 days of salting time.

* $p < .05$; ** $p < .01$; ns: not significant.

lipids ($p < .01$). This result was linked to the whole amount of FAs, higher in the $D \times LW$ group, irrespective if they were saturated (SFAs), monounsaturated (MUFAs) or polyunsaturated fatty acids (PUFAs) ($p < .01$).

As regard Salting Time effect, no significant differences were recorded in FAs profile ($p > .05$).

Volatile compounds profile

The VOCs of 'Cuore di Spalla' are shown in Table 4. Eighty-nine compounds were identified. The identified compounds were clustered in the following chemical families: aldehydes, acids, alcohols, esters, ketones, thiols, terpenes, furans, alkanes and others. The main chemical families were, in order, alcohols $>$ esters $>$ acids $>$ aldehydes and then all the others. About the genotype effect in aldehydes group, only Benzaldehyde showed a significant difference between $CS \times LW$ and $D \times LW$ ($p < .05$). Total acids content was not different between genotypes. However, Butanoic, Octanoic and n-Decanoic acids were lower in $CS \times LW$ than $D \times LW$ ($p < .05$) while Hexanoic acid-2-ethyl was higher in $CS \times LW$ ($p < .05$). $D \times LW$ recorded a higher amount of total alcohols respect to $CS \times LW$. As regard the individual contribution of compounds belonging to this family, significant differences were identified for 3-Octanol, 4-Heptanol-2,6-dimethyl, 1-Hexanol-2-ethyl and Phenylethyl alcohol that were higher in $D \times LW$ ($p < .05$) as well as Phenol-2-methoxy-4-(1-propenyl) ($p < .001$) while Benzenemethanol- α -4-dimethyl, Ethanol-2-(2-butoxyethoxy) and Phenol-4-methyl showed lower values in $D \times LW$ ($p < .05$). For what concern esters, Hexanoic acid ester, 4D-methyl-hexanoic acid ester, Heptanoic acid ethyl ester, Isopropyl dodecanoate and Propanoic acid-2-methyl-1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester recorded higher values in $CS \times LW$ than $D \times LW$ ($p < .05$) whereas Propanoic acid-2-hydroxy and Decanoic acid ethyl esters ($p < .05$) showed lower values. Ketones family did not show any variations in the total amount. $CS \times LW$ obtained the highest values for 2-Heptanone, 6-Methyl-5-hepten-2-one and lowest for 2-Butanone-3-hydroxy and Bicycloheptan-2-one-1,7,7-trimethyl ($p < .05$). In Thiols group only 3-Methylthio-1-propene showed a difference between the genotypes ($p < .05$). Terpenes showed no differences between genotypes. Furans were highest in $D \times LW$ than in $CS \times LW$ apart from 2(3H)-Furanone-5-ethyldihydro ($p < .05$). Alkenes showed highest values of Octane and n-Decane in $CS \times LW$ and lowest for 2,2,4,6,6-Pentamethyl-heptane ($p < .05$). $CS \times LW$

showed highest amount of 1,1-Diethoxy ethane and Formamide-N,N-dibutyl ($p < .05$).

Salting time influenced the levels of Aldehydes. Decanal and Benzaldehyde resulted higher in H group whereas 2-decenal, (E) was higher in L group. Also, in Alcohols, H group showed higher values than L group except for Phenylethyl alcohol ($p < .05$). Octanoic acid ethyl ester in esters family showed a higher value in H group than in L group. For the ketones only Bicycloheptan-2-one-1,7,7-trimethyl was higher in H group ($p < .05$). Thiols follow the same pattern with the highest concentrations in H, significant for 2-Propen-1-thiol and 3-Methylthio-1-propene ($p < .05$). The last families that showed significant differences are Furans where 2(3H)-Furanone-5-ethyldihydro was higher in H group than in L group ($p < .05$).

Sensory analysis

Table 5 shows sensory test results. About genotype effect, $D \times LW$ obtained lower scores ($p < .05$) in lean colour, oiliness, hardness, saltiness, chewiness, juiciness and persistence than $CS \times LW$. Salting time was significant only for saltiness with H group being saltier ($p < .05$). Considering the interaction between Genotype and Salting Time, H level of $D \times LW$ showed higher value of saltiness than L level of $D \times LW$ (5.11 vs 3.85) confirming the greater salt content found in chemical analyses.

Discussion

Effect of genotype

The differences in shoulder weight for different genotypes are both a consequence of differences in the carcase weight (Franci et al. 2005) and of raw commercial cuts proportion (Franci et al. 2003). At the same age, the improved breeds showed a higher *in vivo* performance, consequently, they achieved a greater slaughter weight in lesser time (Acciaioli et al. 2002; Sirtori et al. 2011; Pugliese and Sirtori 2012). In this work, the higher slaughter weight of $D \times LW$ compared to $CS \times LW$ (143 vs 122 kg respectively) affected the products' size.

It is well known the importance of genetic effect also on physical and chemical characteristics of meat (Bonneau and Lebret 2010; Pugliese and Sirtori 2012); in the present research, being LW the maternal basis, the influence of the two paternal breeds was high. The increasing contribution of Duroc on IMF content, reported by other authors (Lebret et al. 2011; Sirtori et al. 2011), was confirmed also in comparison with a

Table 4. Volatile compounds of 'Cuore di spalla' as influenced by genotype and salting time.

	ID	KI	Genotype			Salting Time			G × S	RMSE
			CS × LW	D × LW	p-value	H	L	p-value		
Aldehydes										
Acetaldehyde	MS/KI	650	0.650	0.530	ns	0.650	0.520	ns	ns	0.410
Butanal-3-methyl	MS/KI	884	0.004	0.006	ns	0.004	0.006	ns	ns	0.007
Hexanal	MS/KI	1081	0.280	0.260	ns	0.290	0.260	ns	ns	0.180
Heptanal	MS/KI	1183	0.080	0.060	ns	0.070	0.080	ns	ns	0.060
Nonanal	MS/KI	1392	0.750	0.800	ns	0.870	0.680	ns	ns	0.440
Decanal	MS/KI	1498	0.030	0.030	ns	0.040	0.020	*	ns	0.040
Benzaldehyde	MS/KI	1515	0.390	0.790	*	0.730	0.450	*	ns	0.490
2-Decenal-(E)	MS/KI	1642	0.190	0.100	ns	0.060	0.240	*	ns	0.350
Benzeneacetaldehyde	MS/KI	1646	1.370	1.530	ns	1.430	1.480	ns	ns	2.030
Tetradecal	MS/KI	1910	0.040	0.030	ns	0.030	0.040	ns	ns	0.020
Pentadecanal	MS/KI	2042	0.040	0.030	ns	0.030	0.040	ns	ns	0.030
Hexadecanal	MS/KI	2120	0.700	0.640	ns	0.620	0.720	ns	ns	0.430
9-Octadecenal-(Z)	MS/KI	2693	0.030	0.030	ns	0.030	0.040	ns	ns	0.030
Total			4.560	4.830	ns	4.860	4.530	ns	ns	1.920
Acids										
Propanoic acid-2-methyl	MS/KI	1587	0.140	0.150	ns	0.160	0.140	ns	ns	0.150
Butanoic acid	MS/KI	1637	0.610	1.020	*	0.840	0.800	ns	ns	0.560
Pentanoic acid	MS/KI	1744	2.670	1.780	ns	2.000	2.450	ns	ns	2.560
Pentanedioic acid	MS/KI	1850	0.004	0.005	ns	0.005	0.004	ns	ns	0.003
Hexanoic acid-2-ethyl	MS/KI	1914	0.400	0.280	*	0.320	0.360	ns	ns	0.100
Octanoic acid	MS/KI	2067	0.350	0.490	*	0.420	0.420	ns	ns	0.210
n-Decanoic acid	MS/KI	2279	0.110	0.210	*	0.160	0.160	ns	ns	0.130
Total			6.960	5.680	ns	5.860	6.760	ns	ns	4.960
Alcohols										
Benzenemethanol- α -4-dimethyl	MS/KI	1125	0.020	0.008	*	0.010	0.010	ns	ns	0.020
1-Pentanol	MS/KI	1240	0.050	0.210	ns	0.050	0.200	ns	ns	0.420
1-Butanol-3-methyl	MS/KI	1251	0.090	0.340	ns	0.100	0.320	ns	ns	0.690
3-Heptanol-3-methyl	MS/KI	1295	1.070	1.140	ns	1.110	1.100	ns	ns	0.810
1-Hexanol	MS/KI	1359	0.330	0.310	ns	0.380	0.260	ns	ns	0.300
3-Octanol	MS/KI	1394	0.080	0.170	*	0.180	0.100	*	ns	0.140
Ethanol-2-butoxy	MS/KI	1426	0.450	0.710	ns	0.860	0.130	*	ns	0.630
1-Octen-3-ol	MS/KI	1456	1.090	1.390	ns	1.500	0.990	*	ns	0.870
4-Heptanol-2,6-dimethyl	MS/KI	1509	0.020	0.670	*	0.600	0.080	*	ns	1.010
1-Hexanol-2-ethyl	MS/KI	1510	5.260	12.270	*	12.250	5.280	*	ns	10.900
1-Octanol	MS/KI	1564	0.140	0.170	ns	0.180	0.130	ns	ns	0.130
3-Cycloexen-1-ol-4-methyl-1-(1-methylethyl)	MS		0.470	0.380	ns	0.580	0.260	*	ns	0.700
2-Octen-1-ol	MS/KI	1637	0.030	0.030	ns	0.040	0.020	*	ns	0.020
1-Propanol-3-(methylthio)	MS/KI	1708	0.020	0.060	ns	0.020	0.050	ns	ns	0.110
1-Undecanol	MS/KI	1810	0.020	0.020	ns	0.020	0.020	ns	ns	0.020
Ethanol-2-(2-butoxyethoxy)	MS/KI	1827	0.150	0.070	*	0.120	0.100	ns	ns	0.170
Phenylethyl alcohol	MS/KI	1912	0.830	2.640	*	0.940	2.530	ns	ns	3.770
Phenol-4-methyl	MS/KI	2076	0.170	0.130	*	0.150	0.150	ns	ns	0.040
Phenol-2-methoxy-4-(1-propenyl)	MS/KI	2356	0.008	0.050	**	0.030	0.020	ns	ns	0.040
Total			10.980	19.810	**	19.000	11.790	*	ns	13.900
Esters										
Ethyl acetate	MS/KI	884	0.060	0.090	ns	0.080	0.080	ns	ns	0.050
Propanoic acid-2-methyl ethyl ester	MS/KI	975	0.030	0.040	ns	0.020	0.040	ns	ns	0.050
Butanoic acid ethyl ester	MS/KI	1044	0.100	0.110	ns	0.090	0.120	ns	ns	0.080
Butanoic acid-2-methyl ethyl ester	MS/KI	1064	1.320	1.420	ns	1.030	1.720	ns	ns	1.910
Butanoic acid-3-methyl ethyl ester	MS/KI	1072	2.100	1.960	ns	1.470	2.600	ns	ns	2.870
Pentanoic acid ethyl ester	MS/KI	1142	0.070	0.060	ns	0.050	0.070	ns	ns	0.030
Hexanoic acid ethyl ester	MS/KI	1246	1.670	1.200	*	1.400	1.460	ns	ns	0.540
Hexanoic acid-2-ethyl ethyl ester	MS		0.030	0.020	*	0.020	0.030	ns	ns	0.030
4D-methylhexanoic acid ethyl ester	MS		0.180	0.030	*	0.100	0.100	ns	ns	0.250
Heptanoic acid ethyl ester	MS/KI	1352	0.090	0.050	*	0.060	0.070	ns	ns	0.040
Propanoic acid-2-hydroxy ethyl ester	MS		0.030	0.050	*	0.040	0.050	ns	ns	0.030
Octanoic acid ethyl ester	MS/KI	1441	1.160	1.550	ns	1.600	1.110	*	ns	0.810
Nonanoic acid ethyl ester	MS/KI	1541	0.070	0.050	ns	0.060	0.060	ns	ns	0.060
Decanoic acid ethyl ester	MS/KI	1643	0.180	0.380	*	0.310	0.250	ns	ns	0.190
Ethyl 9-decenoate	MS/KI	1707	0.010	0.020	ns	0.010	0.020	ns	ns	0.020
Linalyl propionate	MS/KI	1657	0.140	0.010	ns	0.130	0.020	ns	ns	0.330
Benzeneacetic acid ethyl ester	MS/KI	1779	0.250	0.210	ns	0.150	0.310	ns	ns	0.360
Isopropyl dodecanoate	MS/KI	1823	0.350	0.180	*	0.200	0.320	ns	ns	0.250
Propanoic acid-2-methyl-3-hydroxy-2,4,4-trimethyl-pentyl ester	MS		0.020	0.020	ns	0.030	0.010	ns	ns	0.040
Propanoic acid-2-methyl-1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	MS		0.110	0.080	*	0.080	0.100	ns	ns	0.060
Hexadecanoic acid ethyl ester	MS/KI	2243	0.160	0.120	ns	0.120	0.160	ns	ns	0.130
Total			8.120	7.540	ns	7.080	8.640	ns	ns	5.510

(continued)

Table 4. Continued.

	ID	KI	Genotype			Salting Time			G × S	
			CS × LW	D × LW	p-value	H	L	p-value	p-value	RMSE
Ketones										
2-Butanone	MS/KI	881	0.340	0.350	ns	0.350	0.340	ns	ns	0.130
2-Propanone	MS/KI	813	0.060	0.050	ns	0.060	0.060	ns	ns	0.030
2-Butanone-3,3 dimethyl	MS/KI	978	0.380	0.350	ns	0.390	0.340	ns	ns	0.160
2-Heptanone	MS/KI	1184	0.250	0.110	*	0.180	0.180	ns	ns	0.180
3-Octanone	MS/KI	1240	0.060	0.007	ns	0.050	0.010	ns	ns	0.150
2-Butanone-3-hydroxy	MS		0.010	0.080	*	0.030	0.060	ns	ns	0.080
3-Octanone-2-methyl	MS/KI	1322	0.120	0.080	ns	0.100	0.100	ns	ns	0.110
6-Methyl-5-hepten-2-one	MS/KI	1341	0.050	0.030	*	0.040	0.030	ns	ns	0.040
2-Pentanone-4-hydroxy-4-methyl	MS/KI	1352	0.060	0.070	ns	0.070	0.040	ns	ns	0.080
Bicycloheptan-2-one-1,7,7-trimethyl	MS		0.030	0.120	*	0.100	0.050	*	ns	0.090
5,9-Undecanediene-2-one-6,10-dimethyl (E)	MS		0.040	0.030	ns	0.040	0.030	ns	ns	0.040
Total			1.440	1.280	ns	1.450	1.260	ns	ns	0.680
Thiols-Sulphur compounds										
2-Propen-1-thiol	MS		0.030	0.030	ns	0.040	0.030	*	ns	0.030
3-Methylthio-1-propene	MS/KI	948	0.110	0.060	*	0.090	0.070	*	ns	0.040
1-Propene-3,3-thiobis	MS		0.150	0.110	ns	0.150	0.100	ns	ns	0.220
Diallyl disulphide	MS/KI	1475	0.340	0.460	ns	0.490	0.320	ns	ns	0.820
Total			0.640	0.630	ns	0.750	0.510	ns	ns	0.940
Terpenes										
Gamma-terpinene	MS/KI	1238	0.100	0.007	ns	0.090	0.010	ns	ns	0.300
L-Linalool	MS/KI	1552	0.160	0.060	ns	0.170	0.050	ns	ns	0.310
Total			0.250	0.070	ns	0.270	0.060	ns	ns	0.590
Furans										
2(3H)-Furanone-dihydro	MS/KI	1602	0.030	0.070	*	0.060	0.040	ns	ns	0.040
2(3H)-Furanone-5-ethylidihydro	MS/KI	1694	0.070	0.050	*	0.070	0.050	*	ns	0.030
5,6-dihydro-H-cyclopenta(b)furan	MS		0.040	0.060	ns	0.040	0.060	ns	ns	0.050
Total			0.140	0.180	*	0.170	0.150	ns	ns	0.070
Alkanes										
Octane	MS/KI		0.080	0.030	*	0.060	0.060	ns	ns	0.040
Propane-2-methoxy-2-methyl	MS/KI	660	0.620	0.750	ns	0.700	0.670	ns	ns	0.310
2,2,4,6,6-Pentamethyl-heptane	MS/KI	954	0.003	0.084	*	0.040	0.050	ns	ns	0.080
n-Decane	MS		0.020	0.010	*	0.020	0.010	ns	ns	0.010
Total			0.100	0.130	ns	0.110	0.120	ns	ns	0.080
Others										
1,1-Diethoxy ethane	MS/KI	889	0.290	0.210	*	0.250	0.260	ns	ns	0.090
Pyrazine-2,5-dimethyl	MS/KI	1316	0.270	0.130	ns	0.260	0.140	ns	ns	0.470
Pyrazine-trimethyl	MS/KI	1391	0.750	0.420	ns	0.850	0.300	ns	ns	1.340
Pyrazine-tetramethyl	MS/KI	1457	0.180	0.110	ns	0.210	0.080	ns	ns	0.350
2,3,5-Trimethyl-6-ethylpyrazine	MS/KI	1491	0.070	0.050	ns	0.090	0.030	ns	ns	0.210
Formamide-N,N-dibutyl	MS/KI	1746	0.250	0.100	*	0.220	0.130	ns	ns	0.300

CS × LW: Cinta Senese × Large White; D × LW: Duroc × Large White; H: 5 days of salting time; L: 3 days of salting time.

* $p < .05$; ** $p < .01$; ns: not significant.

MS: comparison with corresponding mass spectra in NIST05 or Wiley 7 database; KI: matching with reported Kovats Indices.

Peak areas for each analyte were normalised for the appropriate internal standard. The value of each compound is the response ratio to the internal standard to which it is associated.

local breed such as Cinta Senese. IMF, together with moisture and product size, greatly affect final product's salt content, with salt diffusion being positively correlated with water and negatively with IMF (Škrlep et al. 2016). The IMF increase creates barriers to penetration (Martuscelli et al. 2017), as well as the increase in the product's size causes an extension of the diffusion time. The present work confirmed these influences, since higher values of salt were observed in CS × LW products, which were also characterised by lower IMF, higher moisture and smaller size than D × LW.

Colour is one of the most important sensorial characteristics. Pork meat colour is influenced by many intrinsic (e.g. breed, gender, age, type of muscle) and

extrinsic (e.g. feeding, pre-slaughter handling, slaughtering) factors (Tikk et al. 2008). In this research the higher values recorded for Duroc crossbreeds may be attributable to the paternal breed contribution probably for higher slaughter weight and IMF content. Alonso et al. (2015) reported that an increase in the percentage of Duroc genes affected colour parameters, in particular a value; the same authors reported also that Duroc and LW breeds seem to have higher myoglobin content than other breeds. The slaughter weight increase can lead to higher a and b values because of an increase in myoglobin and IMF content, respectively (García-Macías et al. 1996; Latorre et al. 2004; Galián et al. 2009). IMF values also seem to affect L* parameter; Suzuki et al. (2005), on Duroc

Table 5. Sensorial traits of 'Cuore di spalla' as influenced by genotype and salting time.

	Genotype		<i>p</i> -value	Salting Time		<i>p</i> -value	G × S <i>p</i> -value	RMSE
	CS × LW	D × LW		H	L			
Appearance								
Intramuscular fat	3.16	3.05	ns	2.95	3.31	ns	ns	0.82
Lean colour	3.98	3.42	*	3.71	3.62	ns	ns	1.01
Oiliness	3.25	2.73	*	3.05	2.95	ns	ns	0.75
Texture								
Hardness	4.20	3.29	*	3.85	3.53	ns	ns	1.18
Aroma								
Aroma	2.90	3.00	ns	2.78	3.09	ns	ns	0.79
After-flavour	2.16	1.53	ns	1.82	1.88	ns	ns	0.99
Taste								
Saltiness	5.07	4.43	*	5.15	4.34	*	*	0.82
After-taste	1.68	1.22	ns	1.37	1.53	ns	ns	1.00
Rancid	0.13	0.20	ns	0.17	0.17	ns	ns	0.21
Chewiness	3.00	2.62	*	2.79	2.79	ns	ns	0.55
Juiciness	4.45	4.02	*	4.26	4.21	ns	ns	0.47
Persistence	5.21	4.84	*	5.09	4.95	ns	ns	0.56

CS × LW: Cinta Senese × Large White; D × LW: Duroc × Large White; H: 5 days of salting time; L: 3 days of salting time.

A 10-cm unstructured scale was used, whose extremes were 'very low' and 'very high'.

p* < .05; *p* < .01; ns: not significant.

pigs, found that meat having high IMF resulted lighter; similarly, Gjerlaug-Enger et al. (2010), found positive correlation between L* and IMF likely due to the visible fat cells.

Another important parameter for the consumers is meat texture, which depends on raw material (Parolari et al. 1988; Morales et al. 2007; Zochowska-Kujawska 2016). Indeed, the differences between genotypes for raw material characteristics, such as IMF and moisture, could have affected the TPA.

As aforementioned, the inclusion of Duroc breed leads to an increase in IMF (Barton-Gade 1987; Suzuki et al. 2003; Alonso et al. 2015) and, consequently, to an increase of the total lipids content with a possible influence also on fatty acids profile. The higher IMF value found in D × LW could also be due to the different growth rate (Čandek-Potokar et al. 1998) and precocity (Franco et al. 2014) compared to CS × LW. Indeed, at the same age, the slaughter weight was higher in D × LW than in CS × LW (Correa et al. 2006; Galián et al. 2009).

Proteolytic and lipolytic enzymes play an important role in the formation of VOCs. VOCs can result from chemical or enzymatic oxidation of unsaturated FAs and from Strecker or Maillard reactions (Toldrá 1998; Toldrá and Flores 1998). Many authors reported aldehydes as the main family in dry-cured products and as the major contributors of overall aroma, due to their low thresholds (Huan et al. 2005; Ramírez and Cava 2007; Marušić et al. 2014; Lorenzo and Carballo 2015). However, in this study aldehydes were not the most abundant family. This result could be attributable to several factors, with the low level of oxidation present in the products probably being the main one. Indeed, the present products had a lower maturing time

respect to ones reported in other studies (Sunesen et al. 2001; Huan et al. 2005; Ramírez and Cava 2007; Marušić et al. 2014; Narváez-Rivas et al. 2014); a second reason could be the low content of PUFAs from which many aldehydes derive (Ramírez and Cava 2007; Laranjo et al. 2015). The low hexanal value recorded confirms this hypothesis, being Hexanal one of the major oxidation products (Marušić et al. 2014). This compound derives from oxidation of n-6 FAs such linoleic and arachidonic acids (Ramírez and Cava 2007). In this case, Hexanal value suggests a low oxidation for both the genotypes (CS × LW and D × LW). Lastly, considering the great amount of alcohol observed, the lower level of aldehydes can be explained with their reduction to corresponding alcohols by alcohol dehydrogenase (Sunesen et al. 2001). The genotype effect was found only for Benzaldehyde, being more abundant in D × LW products, which had also a higher amount of C18:3 content. This confirms that the origin of Benzaldehyde is mainly attributable to degradation of alpha-linolenic acid (Zhao et al. 2017).

Acids can be produced from hydrolysis, degradation of triglycerides and phospholipids, deamination of amino acids, microbial activity (Huan et al. 2005) and aldehydes oxidation (Škrlep et al. 2016). Long chain acids do not directly affect the flavour of cured products due to their high olfaction thresholds (Muriel et al. 2004). Contrariwise, short chain acids (<6 carbon atoms) have an important role on aroma due to their odour as vinegar, cheese or cucumber (Stahnke 1995) together to low threshold values. CS × LW showed lower values for Butanoic, Octanoic and n-Decanoic acids and higher value of Hexanoic acid-2-ethyl respect to D × LW. However, the low capacity of long

chain acids to influence the aroma may suggest that, between the two genotypes, only the difference found in Butanoic acid can lead to different perceptible flavours. In fact, Butanoic acid is reported to have a strong cheese flavour (García-González et al. 2008).

Alcohols family was the most abundant flavour group in this study agreeing with Muriel et al. (2004) who indicated alcohols as the most important compounds in Iberian dry-cured loin. The formation of alcohols can be attributed to aldehydes degradation, microbial fermentation for branched alcohols and PUFAs oxidation for straight chain ones (Muriel et al. 2004). Alcohols' flavour becomes stronger as their carbon chain increases (García-González et al. 2008). In the present study, only few compounds showed difference between the genotypes, although the total alcohols content was almost double in D × LW.

The esters were formed from the esterification of various acids and alcohols. The contribution of esters to flavour formation is very important, having low odour thresholds (Lorenzo and Carballo 2015) and typically aged meat aroma (Huan et al. 2005) and fruity odour (Zhao et al. 2017). However, genotype appeared to have not affected this group of compounds, being the sporadic differences in individual esters inconsistent.

Ketones, as well as esters, were not affected by genotype and thus, no discrimination is expected on the perception. Nevertheless, Ketones, especially 2-ketones are considered to have a great influence on the aroma of meat having an intense odour (Muriel et al. 2004; Marušić et al. 2014) and a high concentration of ketones is the signal of low product quality (Pastorelli et al. 2003).

No noticeable differences between the genotypes were found for Thiols or Sulphur compounds, as well as for Terpenes and Alkanes. Contrariwise, Furans, that are considered important for desirable aroma due to their low threshold values (Ramírez and Cava 2007), were more present in D × LW. This could be attributed to the higher amount of C18:2 as the formation of these compounds starts from linoleic acid (Muriel et al. 2004).

The different aromatic profile of the two genotypes didn't influence the panellists perception, as olfactory and taste parameters did not show any differences, except for saltiness, confirming the higher salt content in CS × LW recorded with chemical analysis. The genotype effect in the aromatic profile were minimal, especially for compounds with low perceptive threshold values as aldehydes (Pugliese et al. 2010).

As for the other sensorial parameters, colour and IMF seem to contradict the results of the instrumental determination. Carrapiso et al. (2003) concluded that some instrumental measurements are often not particularly useful to assess differences perceived by panellists.

The higher values of juiciness and persistence in CS × LW than D × LW are probably attributable to the higher salt content that could accentuate these perceptions in panellists, as reported by Lorigo et al. (2015) and Lorigo et al. (2016).

Effect of salting time

Generally, the effect of salting time was very low. The high amount of salt found in both treatments (above 8% on wet basis), highlighted the possibility of further reductions to meet the requirements demanded by consumers and EU. Purriños et al. (2012) in a trial with similar salting times on pig shoulder, recorded a percentage of salt content in agreement to the present values. Salting time did not influence physical-chemical parameters (i.e. crude protein, IMF, colour, FAs profile) and the seasoning loss agreeing with other studies (Lorigo et al. 2015; Škrlep et al. 2016). Salt content did not significantly affect texture parameters too (even if H group shows tendentially higher values), contrarily to other researches where higher salt percentages increased hardness values (Lorigo et al. 2015; Škrlep et al. 2016). The changes in texture caused by increasing salt content are correlated to dehydration, that causes meat hardening (Ruiz-Ramirez et al. 2005) and to proteolysis inhibition (Toldrá et al. 1997; Martin et al. 1998). In this research, the same seasoning loss for both salting times was recorded, so, the lack of significant differences is likely due to the high salt percentage in both products and to the reduced gap between H and L groups for these parameters.

The aromatic profile revealed few differences between salting times. Almost all the compounds showed higher values in H than in L group. This result could be attributed to the salt pro-oxidizing and solubilising role, as reported by Purriños et al. (2012), which found higher amounts of compounds derived from lipid oxidation in products with 5 days of salting time when compared to 3 days of salting. The same authors recorded a higher amount of alcohols in the product with 5 day of salting time; it was likely due to the decrease of the water molecules available for alcohols solubilisation. Even in this work, the amount of alcohols was higher in H than L level. Despite the differences found in some important aroma-active

compounds (Benzaldehyde, 1-Octen-3-ol) (García-González et al. 2008; Marušić et al. 2014), the sensory analysis confirmed the absence of substantial differences between the two salting times.

Panellists only perceived a change in saltiness between the two product types, as reported in other works (Corral et al. 2013; Škrlep et al. 2016). However, these authors also reported other differences, probably due to the different products tested or to different seasoning times.

Effect of interaction $G \times S$

The interaction effect was minimal. Only $D \times LW$ genotype showed differences between salting times as regards the salt content and the saltiness parameter of the panel test. Probably the effects of greater size, higher fat content and lower value of moisture in $D \times LW$ respect to $CS \times LW$ caused a differentiation between H and L products. In fact, as already mentioned, the salt diffusion is influenced by size, fat and moisture content of the product. The lower size, lower fat content and higher moisture in $CS \times LW$ allowed also to have a full penetration of salt in L level and then to achieve the same results than H.

Conclusions

The study highlighted the genotype effect, probably linked to the intrinsic characteristics of the two paternal breeds (Duroc and Cinta Senese) that have led to differences in size, fat quantity and physical-chemical parameters of the product. The aromatic profile seems to be only slightly affected, with no or unperceivable differences in the sensorial analysis.

The two salting times led to high salt content in both products, even higher than foreseen. Thus, it seems feasible to produce the 'Cuore di Spalla' with further reduction of salting times with positive influence on health aspects providing that safety issues are guaranteed. Saltiness was the only parameters perceived by panellists and no other substantial differences were found for aromatic profile.

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